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# Behavioral Response of Small Everglades Fish to Hydrological Variation, Predator Cues and Parasites

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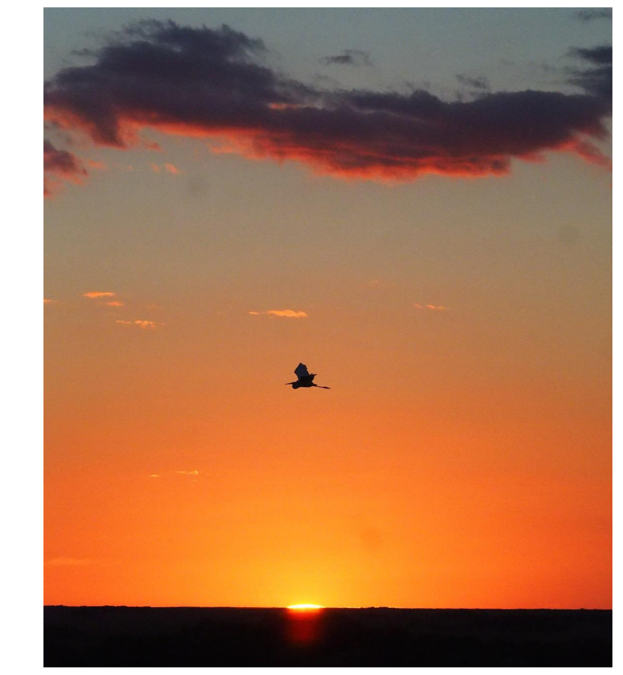
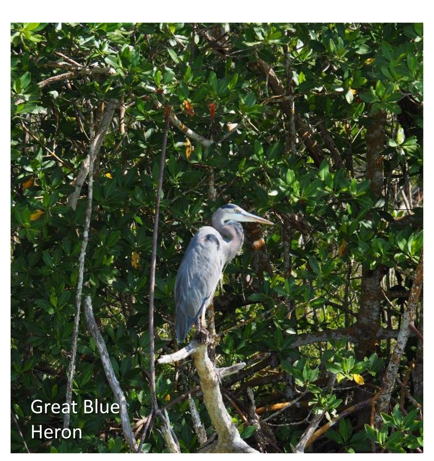


**For videos of our work and process, see J. Matt Hoch's YouTube Channel or:**
**Fish 59 (see below)**
***https://goo.gl/5Mpbx6***
**Fish 41 (see below)**
***https://goo.gl/i9emIw***
**Tracking Process**
***https://goo.gl/EDEGlR***
**Fish capture in drying wetland**
***https://goo.gl/QqeJDi***
**Fish capture**
***https://goo.gl/Ss1n9e***

## Introduction:

Depths in the Everglades fluctuate seasonally. During the dry season, many portions of the Everglades dry completely and are subsequently reflooded during the next wet season. "Short hydroperiod" wetlands dry out for long intervals each year. "Long hydroperiod" wetlands may go years between drying events. Fish increase activity during the periods when water levels change and recolonize these areas quickly in the wet season (Hoch et al, 2015). For example, fish like Eastern Mosquitofish (*Gambusia holbrooki*), may travel long distances during the transition between seasons in order to find the most hydrologically preferable habitat (Trexler et al, 2002). When they are unable to reach deeper water in the dry season, solution holes and remnant pools provide the next best environment (Brandt et al, 2010). These refuges provide habitat for fish and other aquatic life that wading birds depend on as places to forage, and during the dry season they are heavily used while birds build up energy reserves for nesting season. (Frederick et al 2009, Pierce & Gawlik, 2010, Palmer & Mazzotti, 2004; Brandt et al, 2010). While factors influencing the feeding behaviors of wading birds carry scientific weight in their own right, the behavior of their prey is of heightened concern because it can have trait-mediated indirect effects (Gawlik, 2002).

There are many factors that can influence the movement of small fish over the landscape (Bass, 2001). Fish "personality traits", such as boldness, may play a large role in the likelihood of exploring and migrating to new territory (Cote et al. 2010). Boldness is defined as the likelihood to explore new areas of an unfamiliar environment. A "bold" fish will be more likely to take the risk of migrating to an area of unknown habitat quality in order to capitalize on the potential resources in the area. Fish with intrinsically better exploration behaviors are more likely to recolonize newly available wetlands or escape the lethal dry-downs. These behaviors may vary over the water-year or between long and short-hydroperiod wetlands. Another factor that might influence fish behavior are the cues of its predators, including the wading birds. These cues might influence the fish to hide or change course, and may alter migration patterns and therefore impact fish migration patterns (Smith & Belk, 2001). These cues may include visual cues or feces deposits. Parasites are important members of aquatic communities, and many have been shown to affect host behavior. For example, Euhaplorchis sp. alter the behavior of their intermediate host fish to maximize transmission to their definitive hosts, fish eating birds. Others (e.g. Anguillacola sp.) inhibit the normal migratory patterns of their hosts.





## Hypotheses and goals

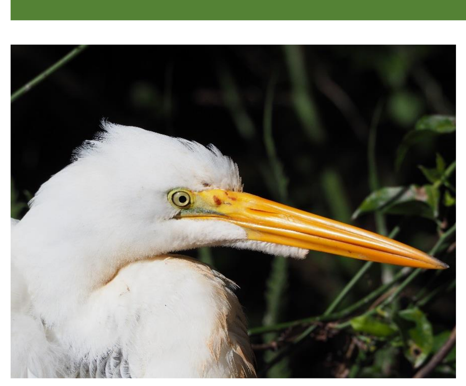
Our goals are to understand factors that affect fish movement across the landscape, including changing water levels, the seasonality of the areas that the fish live in, the presence of predator cues and food motivation. We area also investigating the parasite community to determine whether parasites might influence migration behavior in the community. We performed two experiments.

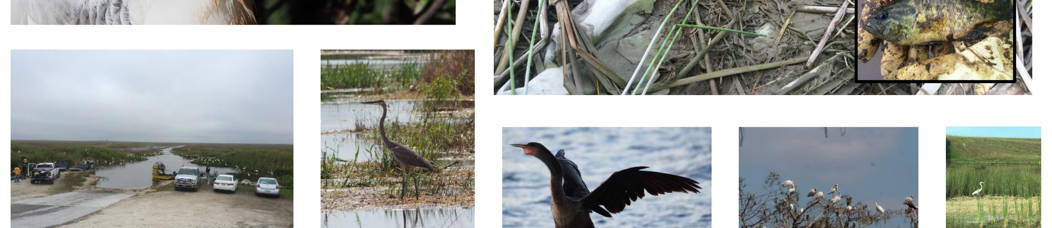
The first tests the hypotheses that:

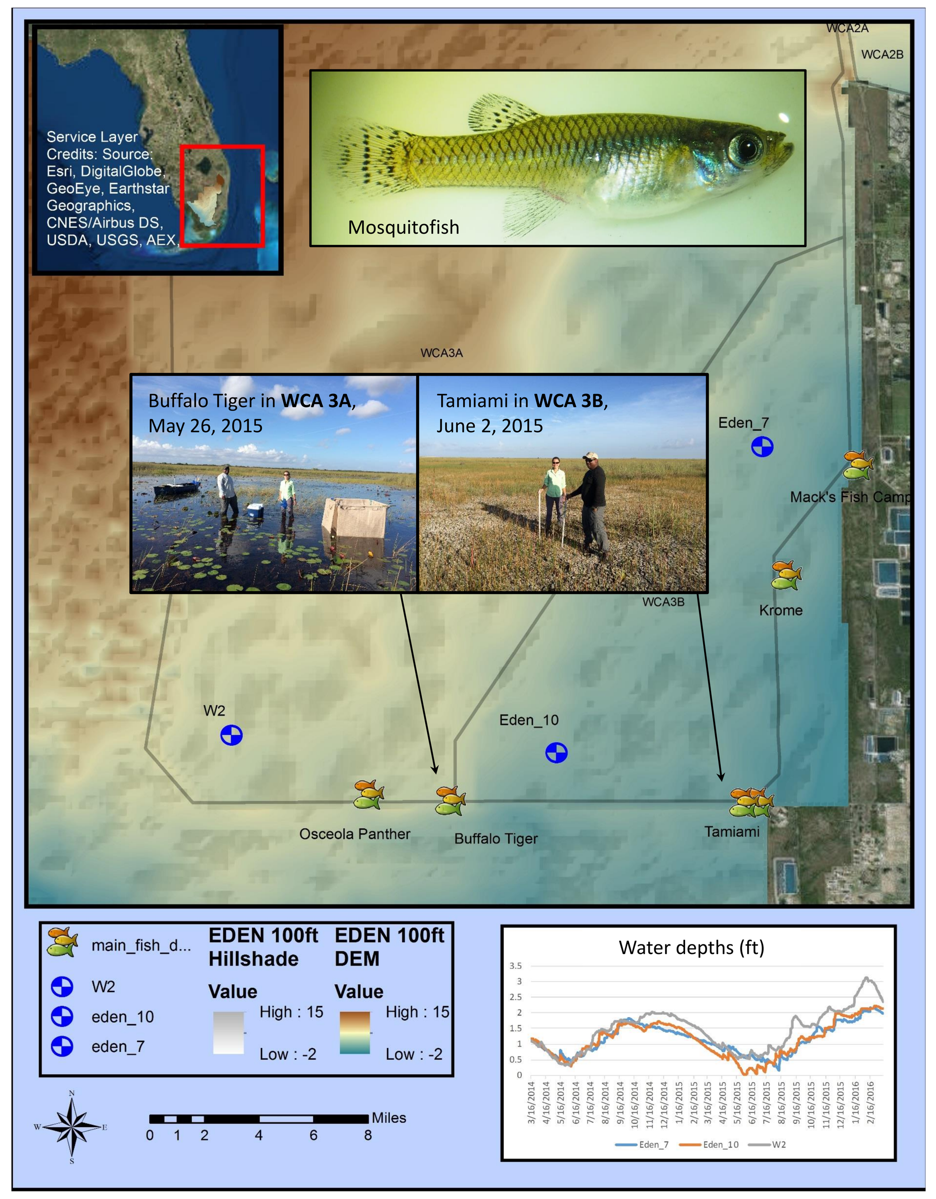
1. Mosquitofish personality and willingness to explore varies based on the hydroperiod environment that the fish has experienced.
2. Personality may shift with season, corresponding to times of year when water rises and falls.

The second tests the hypotheses that:

1. Mosquitofish will spend more time hiding in the presence of a visual predator cue, a bird model.
2. Mosquitofish will be more cautious with the presence of a chemical predator cue, bird feces.
3. Food motivation may overpower risk aversion.







## Materials and Methods

### Field Methods

For the first experiment we sampled Eastern Mosquitofish from five sites between October 2014 and March 2016. Two sites, Buffalo Tiger and Osceola Panther, are located in WCA 3A, a long hydroperiod region that did not dry during the study period. Three sites (Mack's Fish Camp, Krome Ave and Tamiami) are located in WCA 3B, which has a much shorter hydroperiod. All of the WCA 3B sites dried in the summer of 2015 (except for deeper pits and airboat trails). For the second experiment, from August to November 2016, we collected fish only at the Krome Ave and the Mack's Fish Camp Sites.

### Lab methods

For the first experiment, fish were housed in water collected at their site, allowed to acclimate to lab conditions, then filmed swimming in tank filled with the same water. Obstacles prevented the fish from seeing across the tank. Each run began with the fish being placed in "house". After an acclimation period, a door in the house was opened giving the fish access to the tank. The first variable recorded was "latency time," the time the fish takes to exit the house. Eventually, the fish emerged and began to explore. 156 mosquitofish were photographed once a second for twenty minutes while exploring.

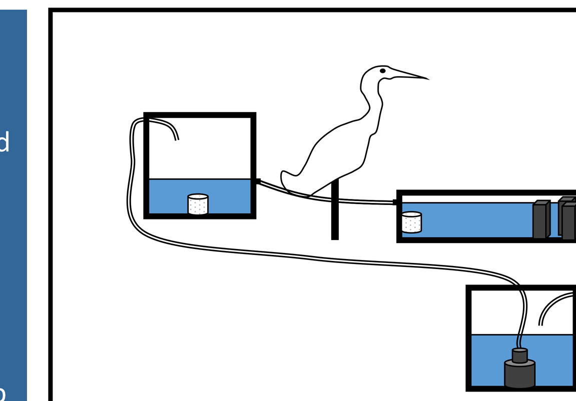
In the second experiment, we used conditioned tap water to ensure there was no bird feces, food or other potential chemical cues already in the water. Fish were allowed to acclimate to lab conditions for two days, but were not fed, so that food motivation was equal for all treatments. The process was similar to the first experiment, except that the tanks were larger and had a recirculating current delivering any cues for the treatment. During the run, fish were exposed to combinations of visual predator cues (a life-size egret replica), chemical predator cues (a slurry containing feces collected from Great Egret, Snowy Egret, Little Blue Heron, Yellow Crowned Night Heron, Double Crested Cormorant, Anhinga, and White Ibis) or fish food (which the fish rapidly approached and consumed in pre-experiment trials). Obstacles surrounded the house, but the half of the tank located next to the bird model was left obstacle free. 72 mosquitofish were photographed once a second for ten minutes while exploring.

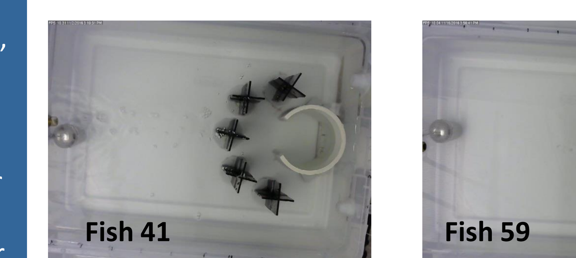
### Computer and Statistical Analysis

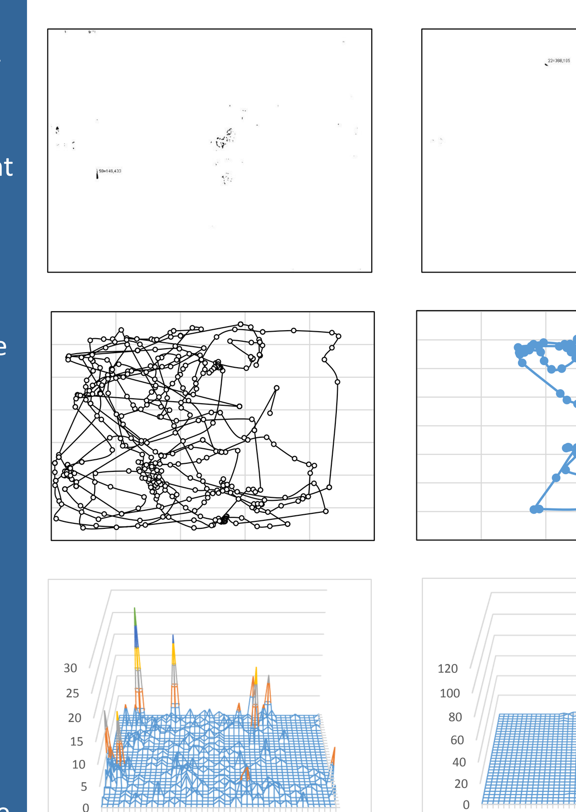
The photos from each run were compiled into "stacks" in ImageJ (Schneider et al., 2012). The Mtrack2 plugin (Stuurman 2008) marked the position of the fish on a coordinate plane in each frame. We determined the total distance the fish swam during the run, the coefficient of variation of its speed, the proportion of the environment it explored, and in the second experiment only, the percent time the fish spent in the open side of the tank. For the first experiment we performed a two-way factorial ANOVAs to identify the effects of the region of origin and the season on the movement variables. In the second experiment we performed three-way factorial ANOVAs to determine the effects of feces cue, the bird model cue, the presence or absence of food, and all their interactions on each response variable.

### Parasitology

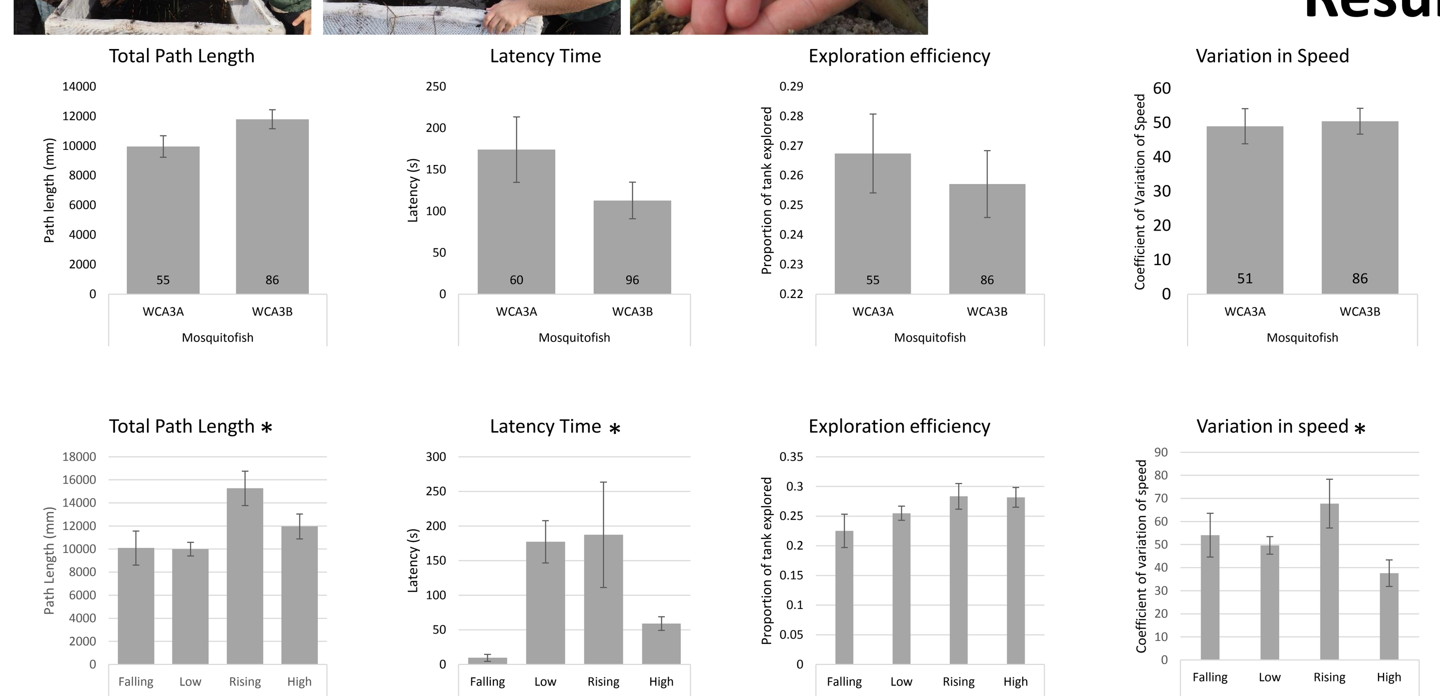
In addition to Eastern Mosquitofish, we also examined Golden Topminnow (*Fundulus chrysotus*), Bluefin Killifish (*Lucania goodei*) and Sailfin Molly (*Poecilia latipinna*) for parasites. Fish were examined with a stereomicroscope for ectoparasites. Fins and eyes were removed and examined individually. The body cavity was opened ventrally, and all visceral organs (heart, liver, spleen, gallbladder, digestive tract, gonads) examined for endoparasites. Individual organs were pressed between glass plates and examined with the stereomicroscope. Gut contents of each fish were also examined for parasites. All parasites were identified using standard parasite identification keys.



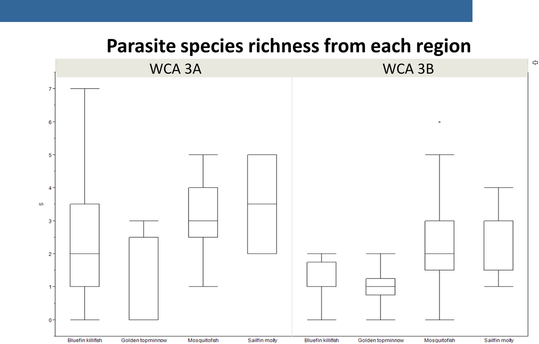


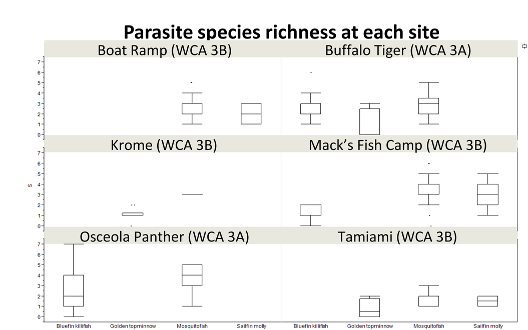
## Results



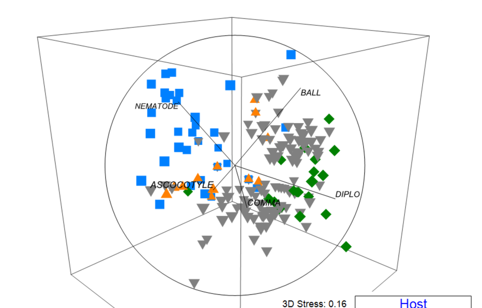

**Left figures**: We compared our exploration variables for using ANOVAs. Mosquitofish captured at the different sites did not vary significantly for our variables. Mosquitofish swam farthest when waters rose, had greatest latency when waters were low or rising and had the greatest variability in their speed when waster were rising. Significant differences of these variables are marked with "*" on the chart.



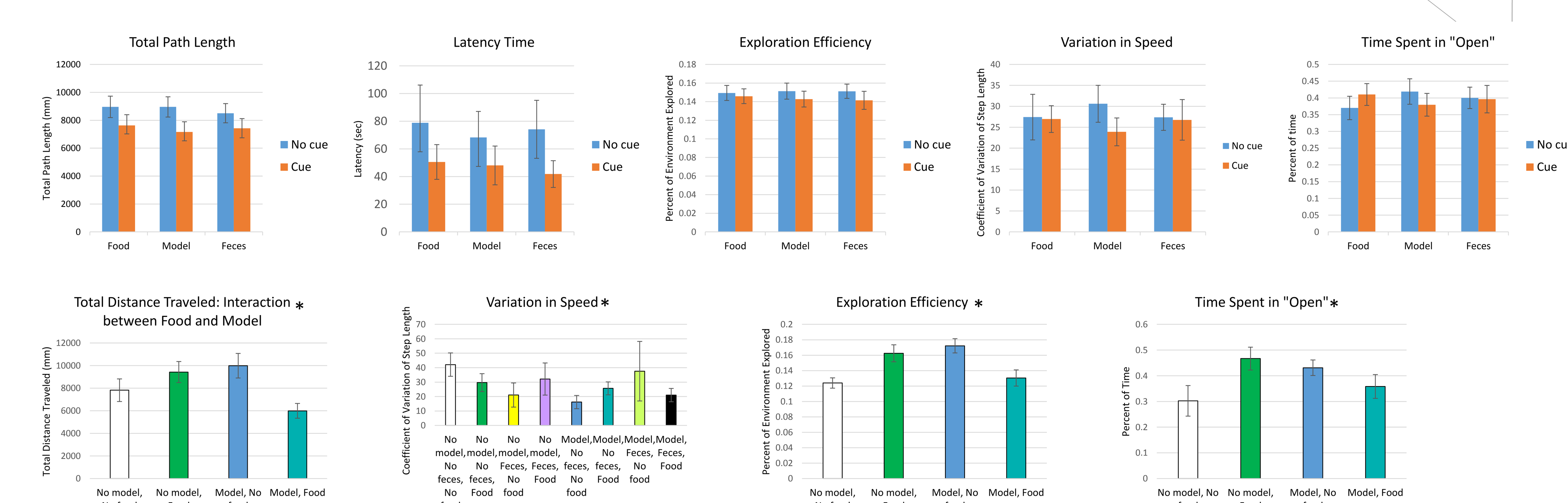

**Above figure**: Quartile boxplots showing parasite species richness found in each host fish in each region. Parasite diversity did not differ between regions.



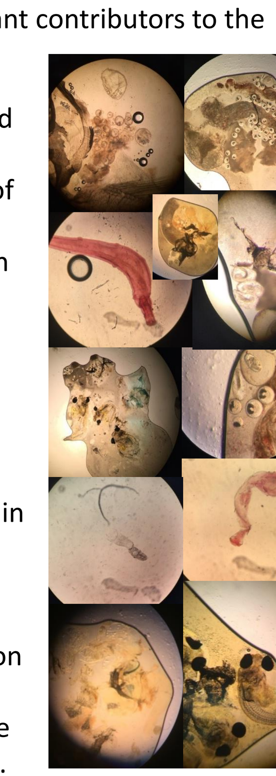

**Above figure**: Quartile boxplots showing parasite species richness found in each host fish at each site. Both site and species affected parasite community, but species more so.




**Left figure**: MDS plot (proximity of points is proportional to the similarity of their communities) showing similarity of parasite communities found in four fish species. ANOSIM showed that site of capture and host species were the most important contributors to the pattern.




**Left figures**: We compared our exploration variables for using ANOVAs. None of the cues contributed significantly to variation in our variables (top row). However, we saw significant effects of the interactions model and food presence on total path length, search efficiency and time spent in the open. There was a significant effect of the interaction between all three variables on variation in speed (bottom row).
**Right images:** Some of the parasites we found in fish.



## Discussion

In the first experiment, there were clear effects of seasonality of the water-year on mosquitofish behavior. The observed behavioral differences were consistent with reports from the field (Hoch et al. 2015), in which fish activity drastically increases during hydrological change. Fish in both regions seem to respond to this change and adjust their behavior to increase the likelihood of surviving dry periods and migrating to exploit resources after water levels rise. They swam farther, were more efficient and changed speeds more often. They also had longer latency during those time periods, indicating that they might be adjusting their levels of risk tolerance/risk aversion during these periods. We noticed that water quality was drastically reduced during the periods of low water and that the fish seemed to respond to it negatively. That response was part of the motivation for the second experiment.

In the second experiment, latency times were shorter for all the cues, which is somewhat counterintuitive since we predicted that the presence of the predator cues would make the fish less likely to explore. Another unexpected result was that the chemical cues from the feces had little effect on the fishes' behavior. This could be because chemical cues are long-lasting in the Everglades dry season. Water quality in remnant pools is typically very poor and is filled with a variety of highly concentrated chemical cues. If the cues are persistent, the fish may not use them as a reliable predator cue.

The fish explored the largest percentage of the environment with the bird model present, so it could be argued that the bird model had no effect on fish behavior. However, the fish also had the lowest variation in speed with the model present, indicating that they were either uniformly stationary or constantly moving. Perhaps the fish were aware of the model's presence, and modified their behavior in accordance. We noticed that the fish spent a significant amount of time around the edges of the tanks and less time around the obstacles. From the fishes' perspective, this may have served as enough of a shelter.

The fish spent the most time in the open when the only cue was food. This implies that the presence of food made fish more likely to engage in foraging behavior outside of the sheltered area. This is consistent with previous studies of fish behavior and predator evasion (Pierce & Gawlik, 2010; Trexler, et al, 2002). They spent considerably less time in the open when both food and bird model were present, perhaps influencing risk aversion. In the context of the Everglades, this means that food presence makes fish more likely to take risks and expose themselves to predators, unless they are aware of the predator's presence. Knowing that the predator is close by may cause them to take evasive action even when there is food present. Fish in the wild may be cognizant of the presence of a predator and make decisions to avoid that predator as much as the environment permits. This could impact the success with which wading birds forage during dry periods. It may also cause fish to leave hydrated areas earlier. This could impact the success with which the fish escape drying areas and therefore the success of wading bird foraging.

Each fish species harbored a distinct parasite community, which differed slightly among sites. We observed that collections from sites with the greatest number of birds or during periods of falling water were associated with diverse and abundant parasites. Many of the observed parasite taxa are known to change host behavior, but further analysis is necessary to determine how they affect the fish species here. Because the fish are a critical food source of the birds, parasites may be a very important component of the Everglades ecosystem.

## Acknowledgments

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